DEPARTMENT OF
ANIMAL & AVIAN SCIENCES

UNIVERSITY OF MARYLAND
College of Agriculture and Natural Resources

Genome Editing in Agricultural Animals: Opportunities and Challenges

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USDA ARS

RENOVATE Biosciences

Overview of the talk

- Rationale for performing genome editing
- Genome editing using CRISPRs
- Path forward

Rationale for performing genome editing in livestock

Animal Biotechnologies in Context

SCIENTIFIC CONTINUUM

Genetic Modification

- Mass selection
- Pedigree selection
- Marker-assisted selection
- **Transgenics** (1980s) (**GE Animals**)
- Genome-wide selection
- **Gene Editing** (2000s) "Precision Breeding"

Objective

Change Genetic Makeup

Source: Dr. Diane Wray-Cahen

Genetic bottleneck associated with conventional breeding

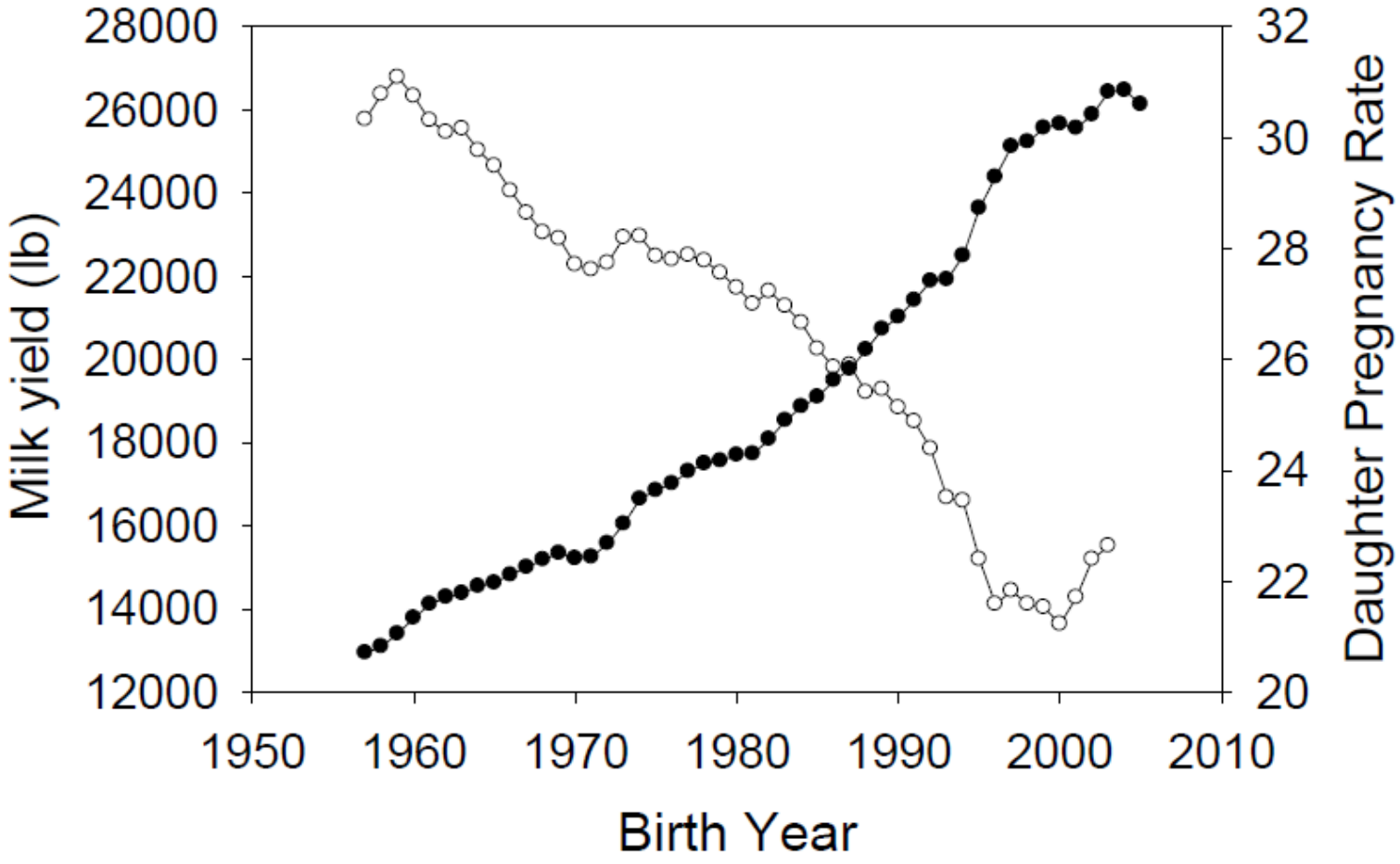
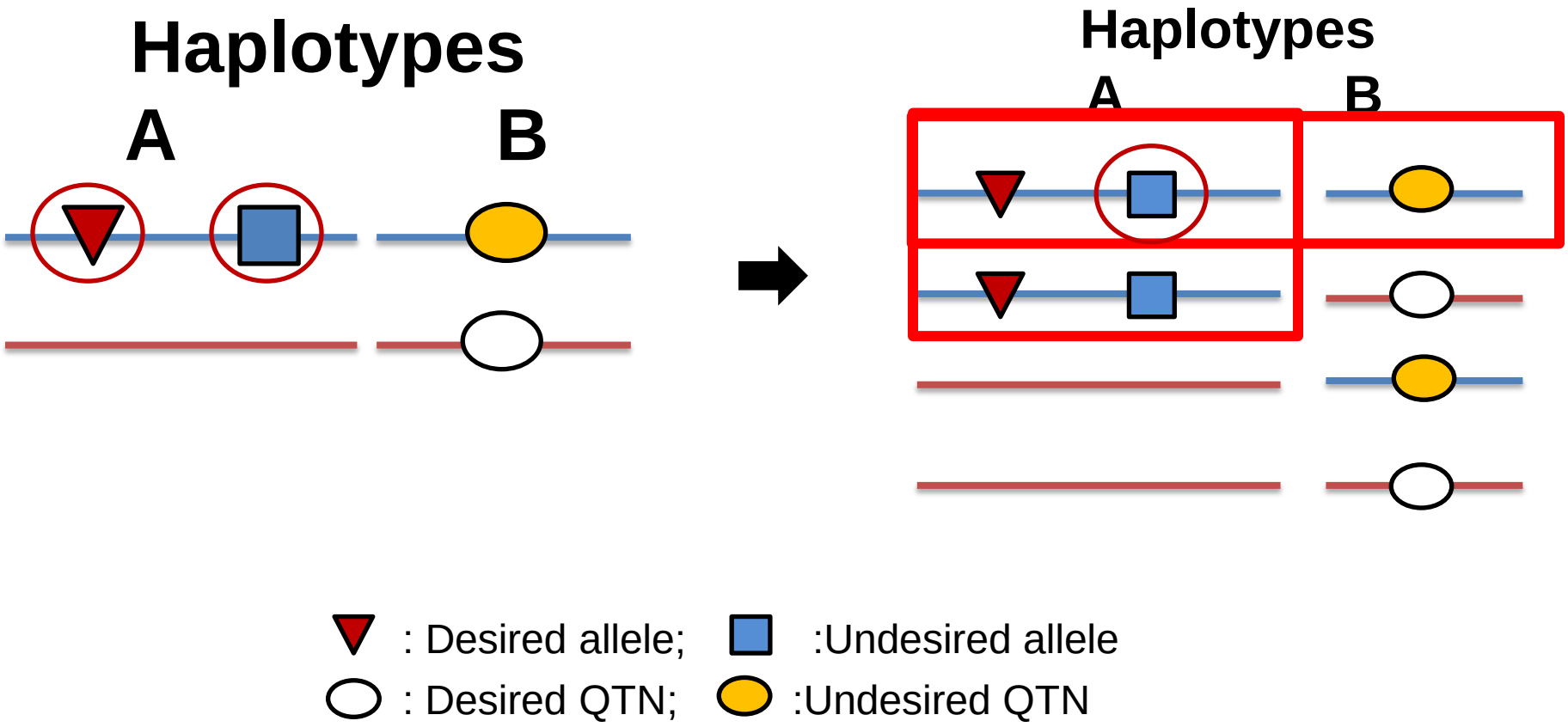


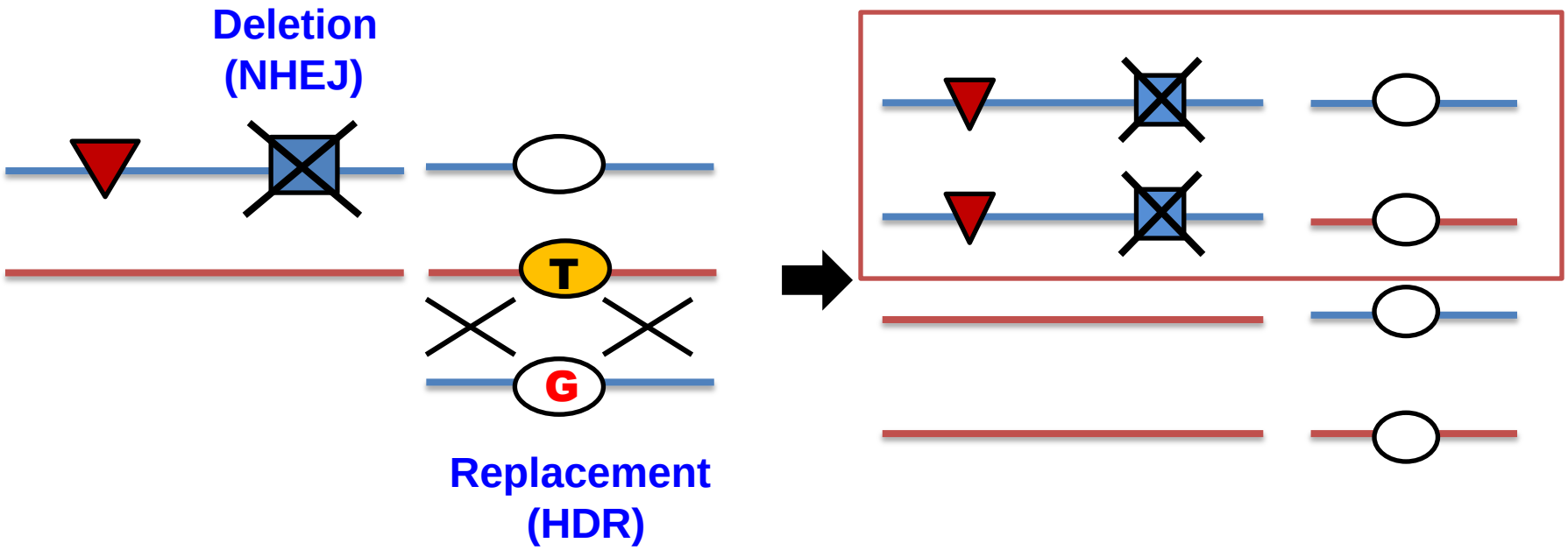
Figure 1. Trends in milk yield (●) and Daughter Pregnancy Rate (○) for US Holsteins. Data are from USDA-ARS Animal Improvement Programs Laboratory, February 2007 (available at http://aipl.arsusda.gov/ARSWeb/eval/summary/trend.cfm).

Genetic bottleneck associated with conventional breeding



Telugu et al., *NIB*, 2017

Rational Selection via Genome editing to accelerate genetic selection

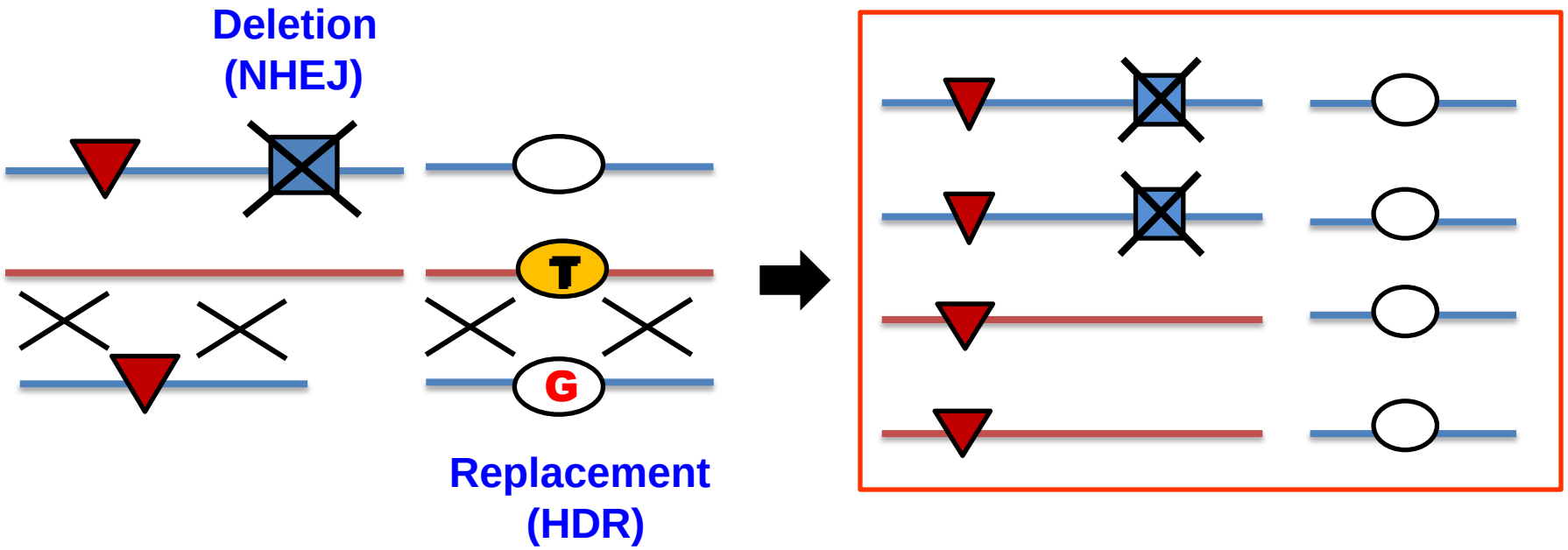


: Desirable allele; :Undesirable allele

: Desirable QTN; :Undesirable QTN

Telugu et al., *NIB*, 2017

Rational Selection via Genome editing to accelerate genetic selection



: Desirable allele; :Undesirable allele

: Desirable QTN; :Undesirable QTN

Telugu et al., *NIB*, 2017

Rationale for Genome editing over Conventional Breeding

- Separate “linked” genes
- Overcome otherwise low heritability
- Increase precision and efficiency of introducing desirable traits (*conventional breeding is random*)
- Introduction of traits not available via conventional breeding

Engineering novel traits
Project: Eliminating boar taint

Boar taint

- Boar taint is an offensive off order and taste found in uncastrated male pigs.

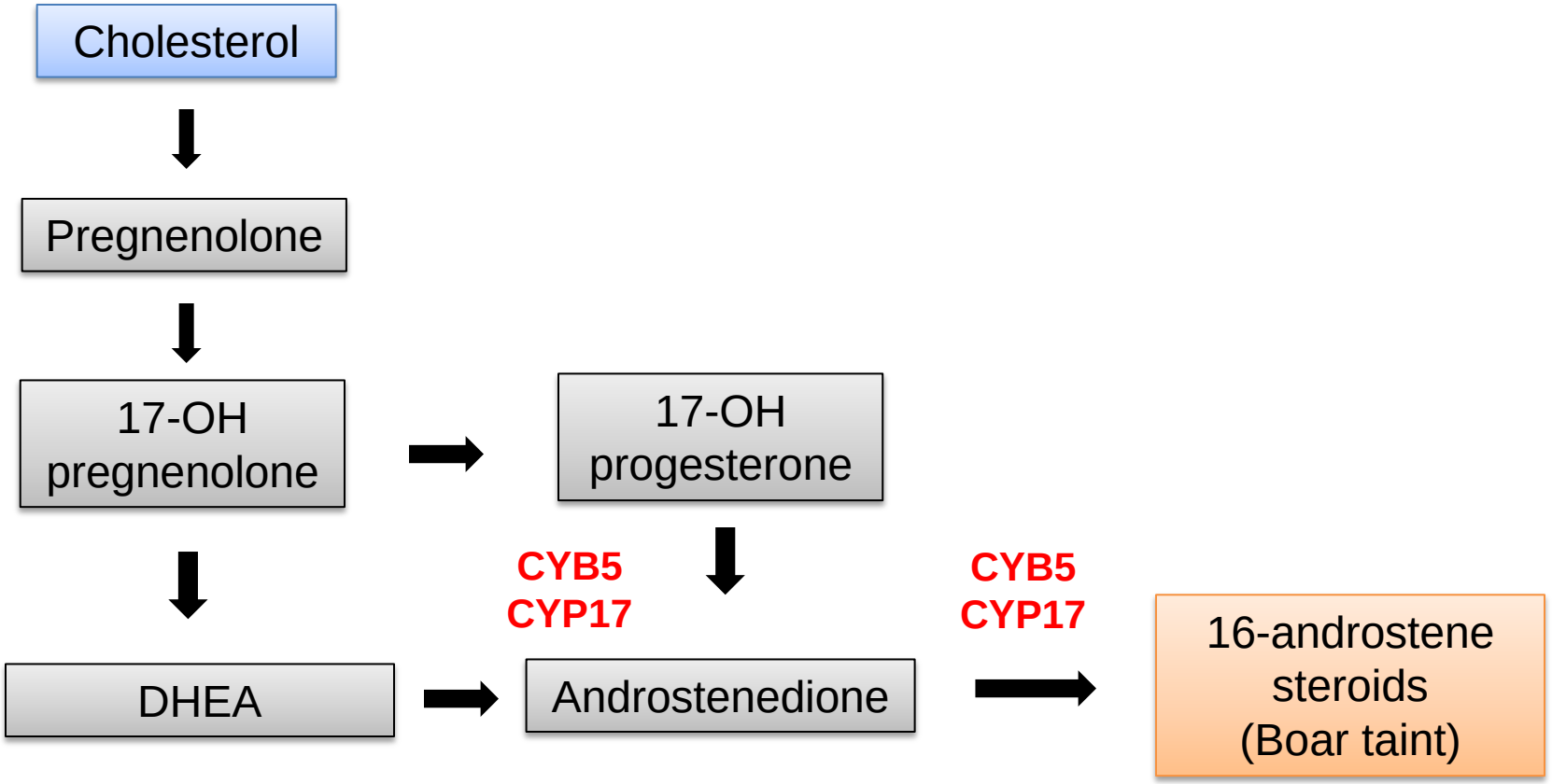
- The major compounds responsible for boar tainted are **androstenone** and skatole, and both compounds are accumulated in fat.

- The goal of this study is to reduce **6-androstenone** production

QTL analysis

- A rare polymorphism in the porcine ***CYB5*** gene just upstream of the translational start site results in **decreased** production of CYB5 and decreased synthesis of androstenone (Peacock et al., 2008).

Boar taint etiology



Comparative genomics

Steroid binding pocket of CYB5A

```
            1          11         21         31         41
Rat CYB5    MAEQSDKDVK YYTLEEIQKH KDSKSTWVIL HHKVYDLTKF LEEHPGGEEV
human CYB5  MAEQSDEAVK YYTLEEIQKH NHSKSTWLIL HHKVYDLTKF LEEHPGGEEV
pig CYB5    MAEQSDKAVK YYTLEEIQKH NNSKSTWLIL HHKVYDLTKF LEEHPGGEEV
```

Steroid binding pocket of CYP17A1

```
            80         90         100        110        120
Human       QLAKEVLIKK GKDFSGRPQM ATLDIASNNR KGIAFADSGA HWQL
Pig         QLAKEVLLKK GKEFSGRPRV MTLDILSDNQ KGIAFADHGT SWQL
Rat         QLAREVLIKK GKEFSGRPQM VTQSLLSDQG KGVAFADAGS SWHL
```

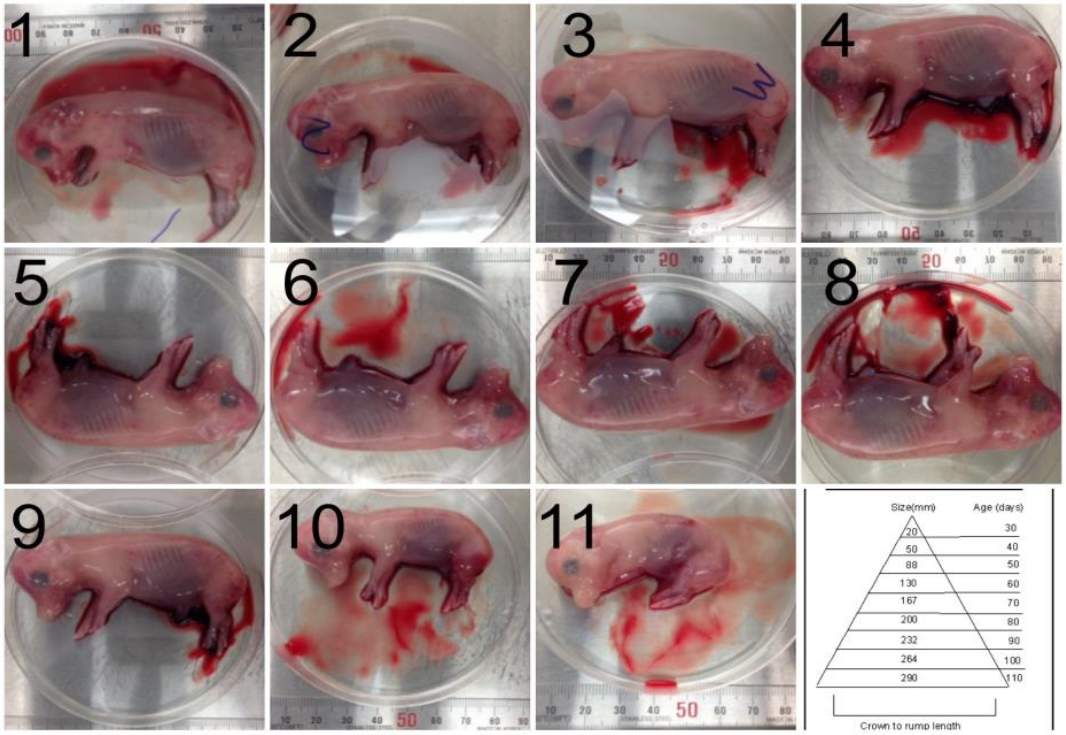
In vitro mutagenesis screen

CYB5 mutations with CYP17 mutations	17OHP	DHEA	16A	16A/DHEA ratio
R52M +L102Q	1.174	0.699	0.607	1.032
R52M +I112V	1.257	0.566	0.282	0.567
R52M +L102Q/I112V	1.500	1.162	0.750	0.282
R52M/D103S	1.282	0.761	0.457	0.600
R52M/S106A	1.484	0.529	0.616	1.167
R52M/NQ108QG	1.176	0.861	0.563	0.653
N62S + D103S	0.897	1.166	0.912	0.787
N62S + 104L	0.904	1.317	1.760	1.484
N62S + S106D	1.252	0.071	0.399	2.042
N62S +L102Q/I112V	1.032	0.963	0.748	0.765
R52M+N62S/D103S	1.195	0.827	0.534	0.645
R52M+N62S/S106A	1.437	0.546	0.799	1.462
R52M+N62S/NQ108QG	1.130	0.877	0.771	0.881
R52M+N62S + L102Q/D103S/I112V	1.130	0.839	0.333	0.426
R52M/G57R/N62S/T70S + L102Q/D103S/I104L/NQ108QG/I112V	1.536	0.257	0.503	1.979
G57R + D103S	0.950	1.085	0.905	0.836
G57R + NQ108QG	0.833	1.255	1.231	0.983
T70S + D103S	0.947	1.087	0.937	0.863
T70S + NQ108QG	0.855	1.221	1.201	1.180
N21K + D103S	1.132	0.835	0.490	0.585
L28V + D103S	1.068	0.924	0.643	0.693
N21K/L28V + D103S	1.110	0.867	0.588	0.677

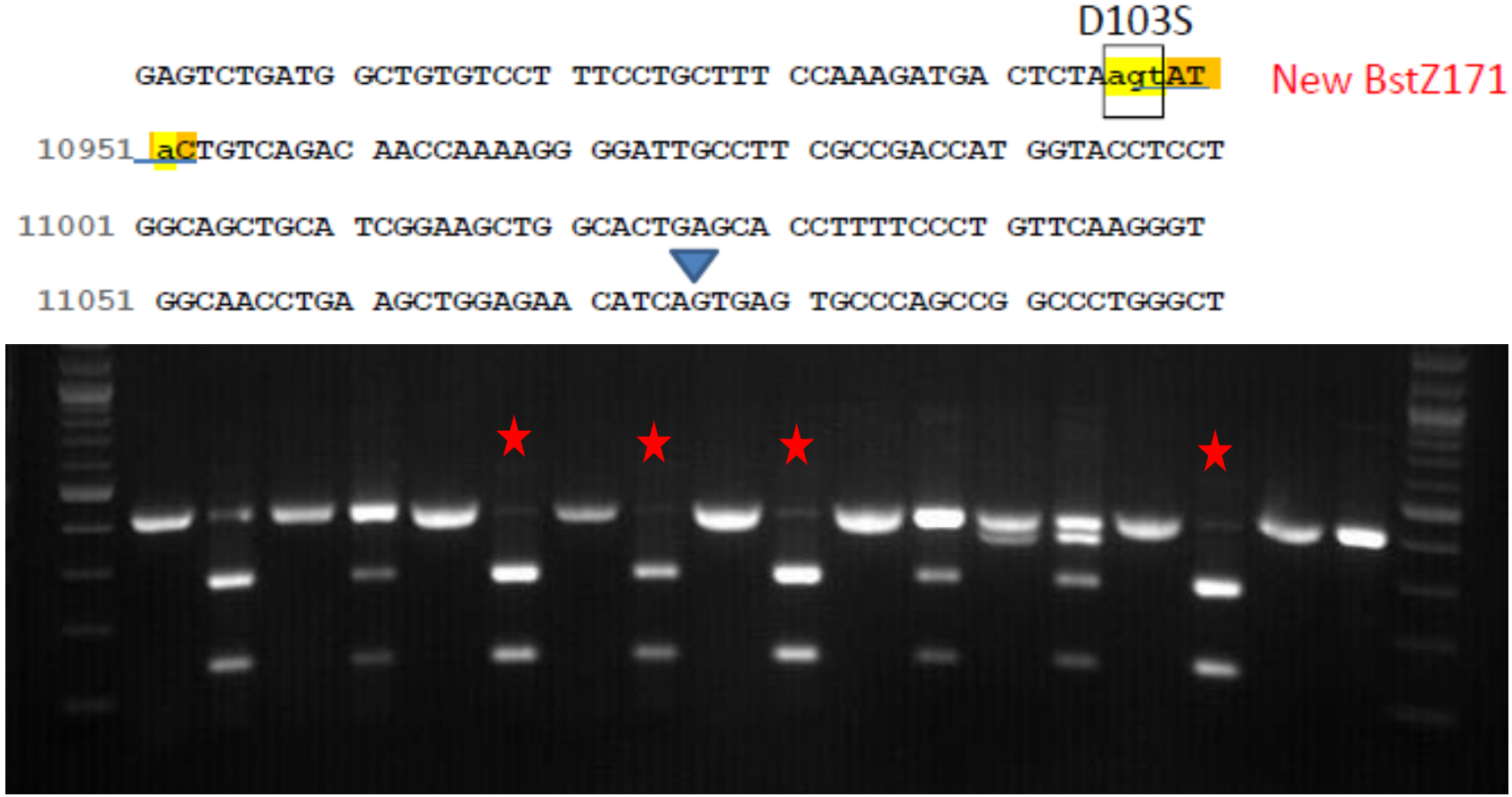
Efficiency of generating genetically engineered pigs by SCNT and CRISPR/Cas9 system

Cell sources	No. recipients	No. pregnancy	No. delivered	No. piglets (fetuses)
KI-CYP17a1	1	1/1 (100)	-	**(11)**

* Cloning efficiency that was obtained by total no. fetus / total no. transferred embryos



Screening of CYP17A1 targeted fetuses by restriction enzyme (BstZ17I) digestion



P: PCR product
D: Digest with BstZ17I

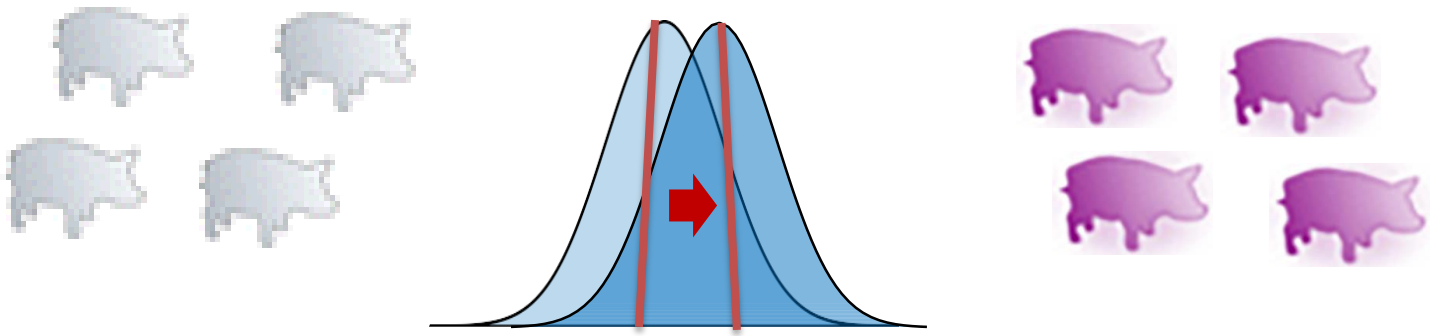
Boar taint project
Summary

- Edit *CYB5* locus on *CYP17*^{mut} background.
- Perform NT with the *CYB5* and *CYP17* double mutants and Wild type controls
- Screen for steroid profile at weaning and at puberty

II. Engineering novel traits
Applied technologies

Regulatory bottleneck

GENETIC IMPROVEMENT



Reference Population (Target for genome editing)

dT (time)

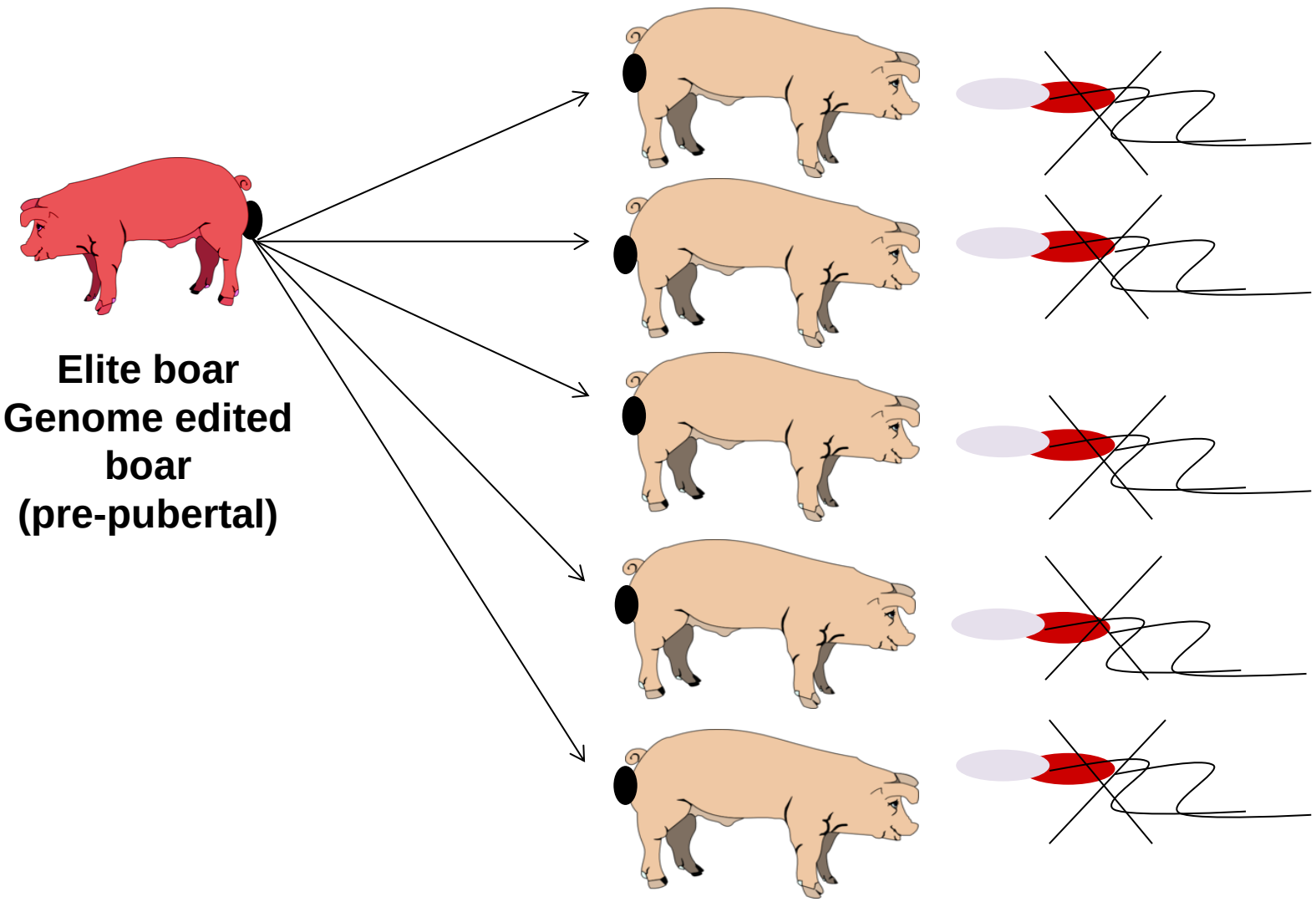
Current Population >Genetic merit (non-edited)

How to close the genetic lag of the edited population given the long generation interval?

Objective:

1) Generate Surrogate sires; and

2) Germ cell transplantation to propagate/ disseminate genetics



I. Generating knockout animals

Project: Generation of germ cell ablated pigs

www.nature.com/scientificreports

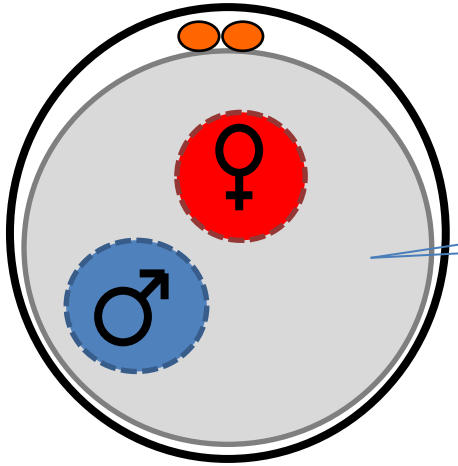
SCIENTIFIC REPORTS

Generation of germline ablated male pigs by CRISPR/Cas9 editing of the *NANOS2* gene

Ki-Eun Park[1,2,3,*], Amy V. Kaucher[4,*], Anne Powell[2], Muhammad Salman Waqas[4], Shelley E.S. Sandmaier[1,2], Melissa J. Oatley[4], Chi-Hun Park[1,2], Ahmed Tibary[4], David M. Donovan[2], Le Ann Blomberg[2], Simon G. Lillico[5], C. Bruce A. Whitelaw[5], Alan Mileham[6], Bhanu P. Telugu[1,2,3] & Jon M. Oatley[4]

Knockout

Generation of *NANOS2* knockout germ cell free animals for SSC transplantation

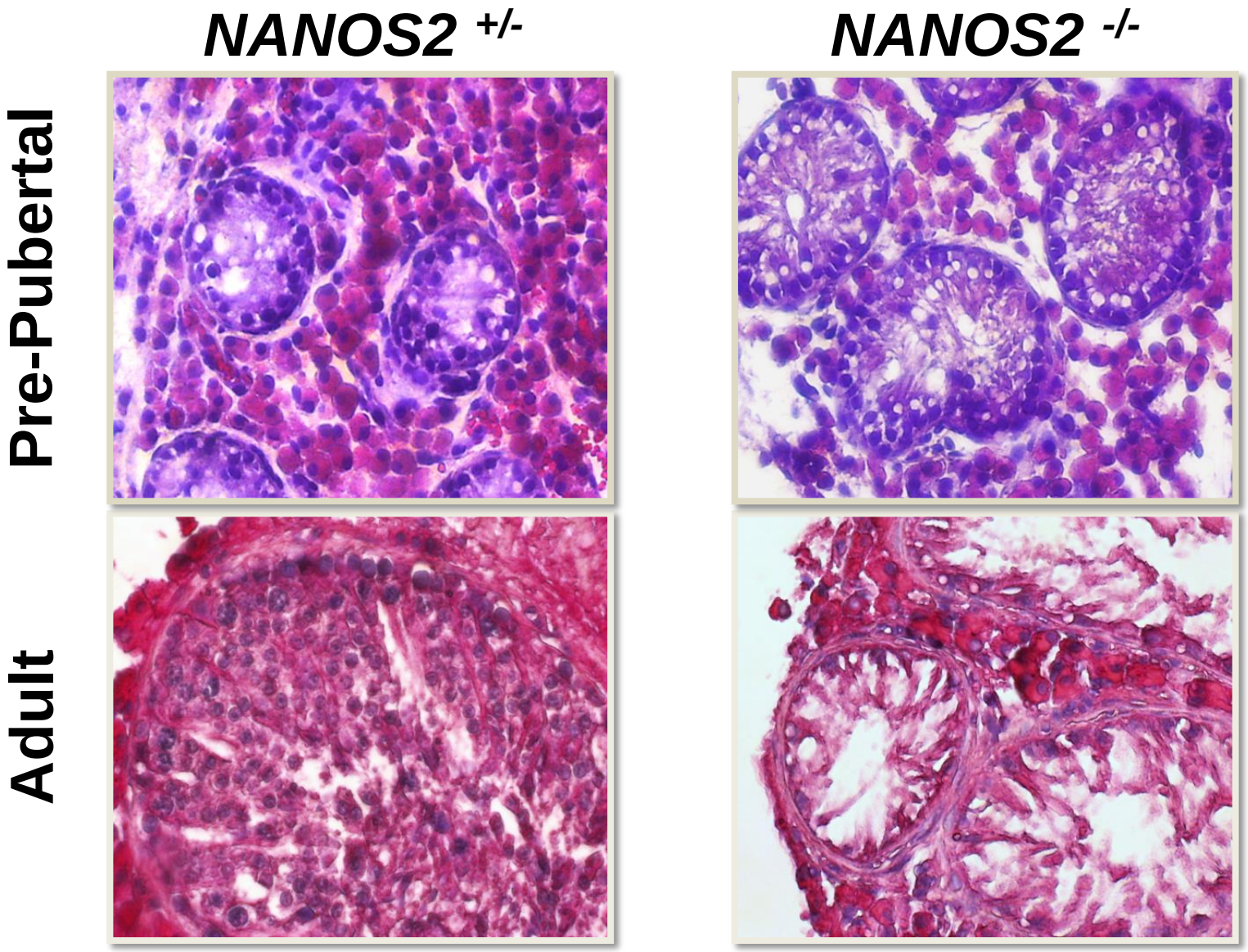


CRISPR/cas9
+ sgRNA mRNA

surg date	DOB	piglet#	sex	
9/23/2014	1/15/2015	1	male	Mosaic
9/23/2014	1/15/2015	2	male	homozygous KO
9/23/2014	1/15/2015	3	female	Heterozygous
9/23/2014	1/15/2015	4	female	homozygous KO
9/23/2014	1/17/2015	10	male	Bi-allelic
9/23/2014	1/17/2015	11	male	Bi-allelic
9/23/2014	1/17/2015	12	male	homozygous KO
9/24/2014	1/18/2015	20	male	Bi-allelic
9/24/2014	1/18/2015	21	male	homozygous KO
9/24/2014	1/18/2015	22	male	Bi-allelic
9/24/2014				
9/24/2014	1/18/2015			homozygous KO
~~9/24/2014~~	~~1/18/2015~~			~~Bi-allelic~~
~~9/24/2014~~	~~1/18/2015~~			~~homozygous KO~~
9/24/2014	1/18/2015			homozygous KO
9/24/2014	1/18/2015		male	Bi-allelic
9/24/2014	1/18/2015	29	male	Bi-allelic
9/24/2014	1/18/2015	30	male	homozygous KO
~~9/24/2014~~	~~1/18/2015~~	~~0~~	~~male~~	-

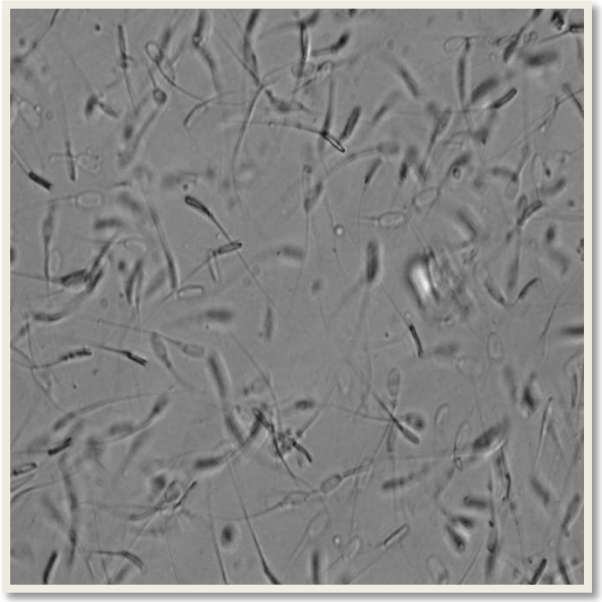
All piglets edited!!

Seminiferous tubule morphology

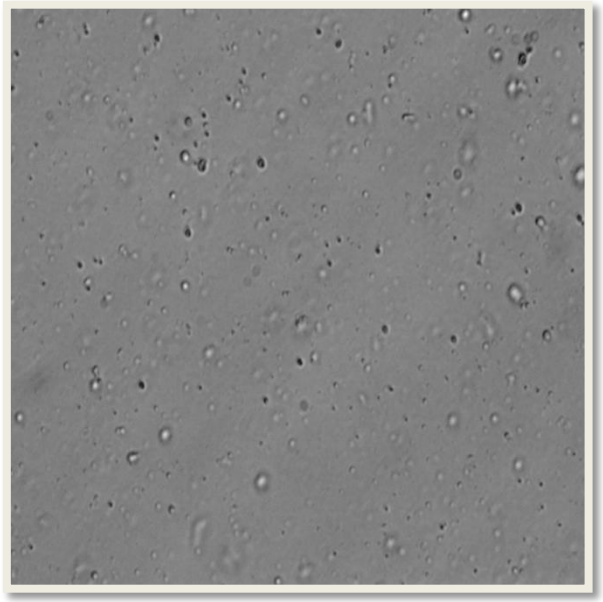


Lack of sperm production in *NANOS2* null males

***NANOS2* $^{+/-}$**



***NANOS2* $^{-/-}$**



Semen collected by manual stimulation

Summary

- The role of *NANOS2* in male fertility is conserved in pigs (other livestock ?)
- Females knockout for *NANOS2* are fertile

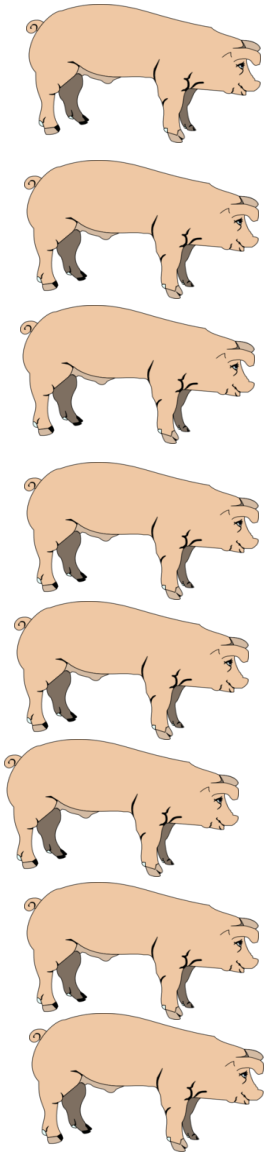
Future goals:

- Expanded the *NANOS2* null boar by SCNT (n=6)
- SSC transplantations were performed.

Future directions

Genome editing in embryos followed by nuclear transfer

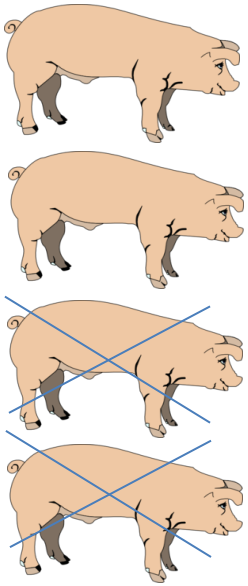
Rationale: Diminished returns



Donor animals

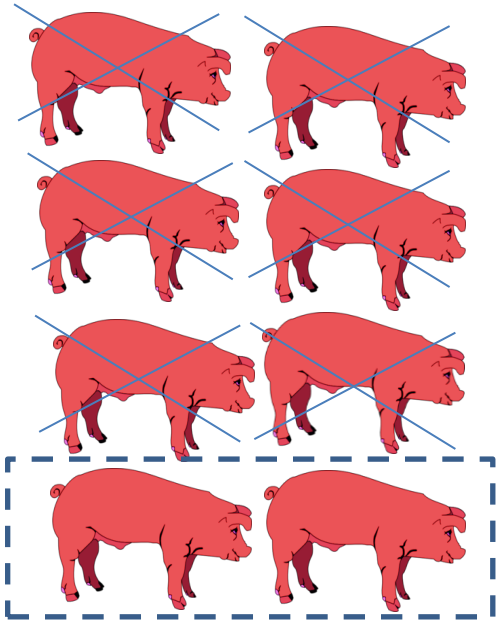
Embryo Recovery/ injections

Embryo transfer



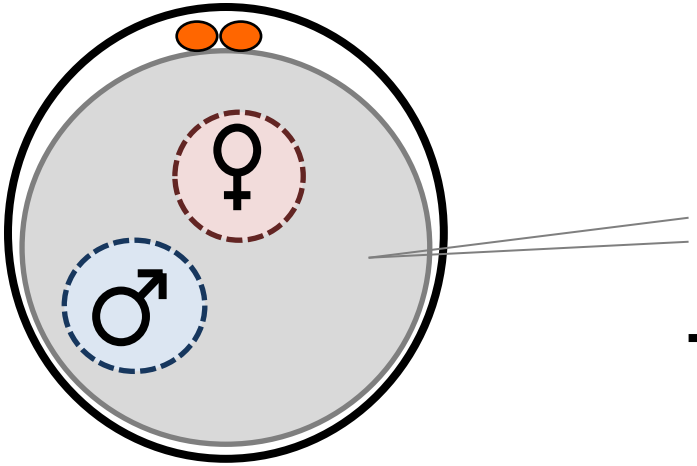
Surrogates

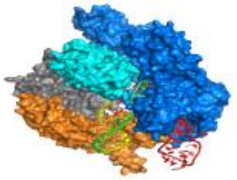
Delivery



Edited animals

Outline

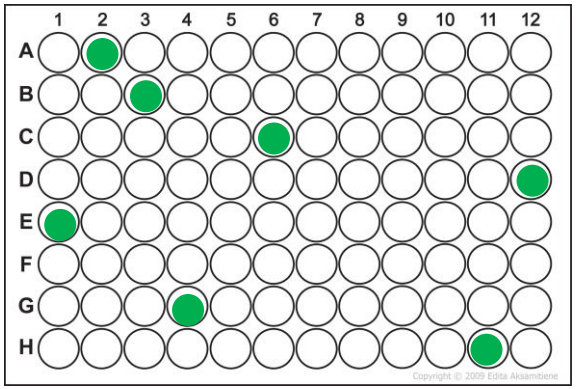


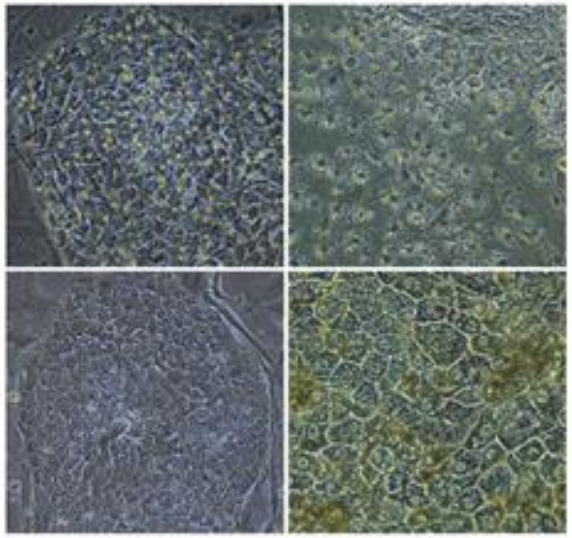


Cas9 + sgRNA RNP

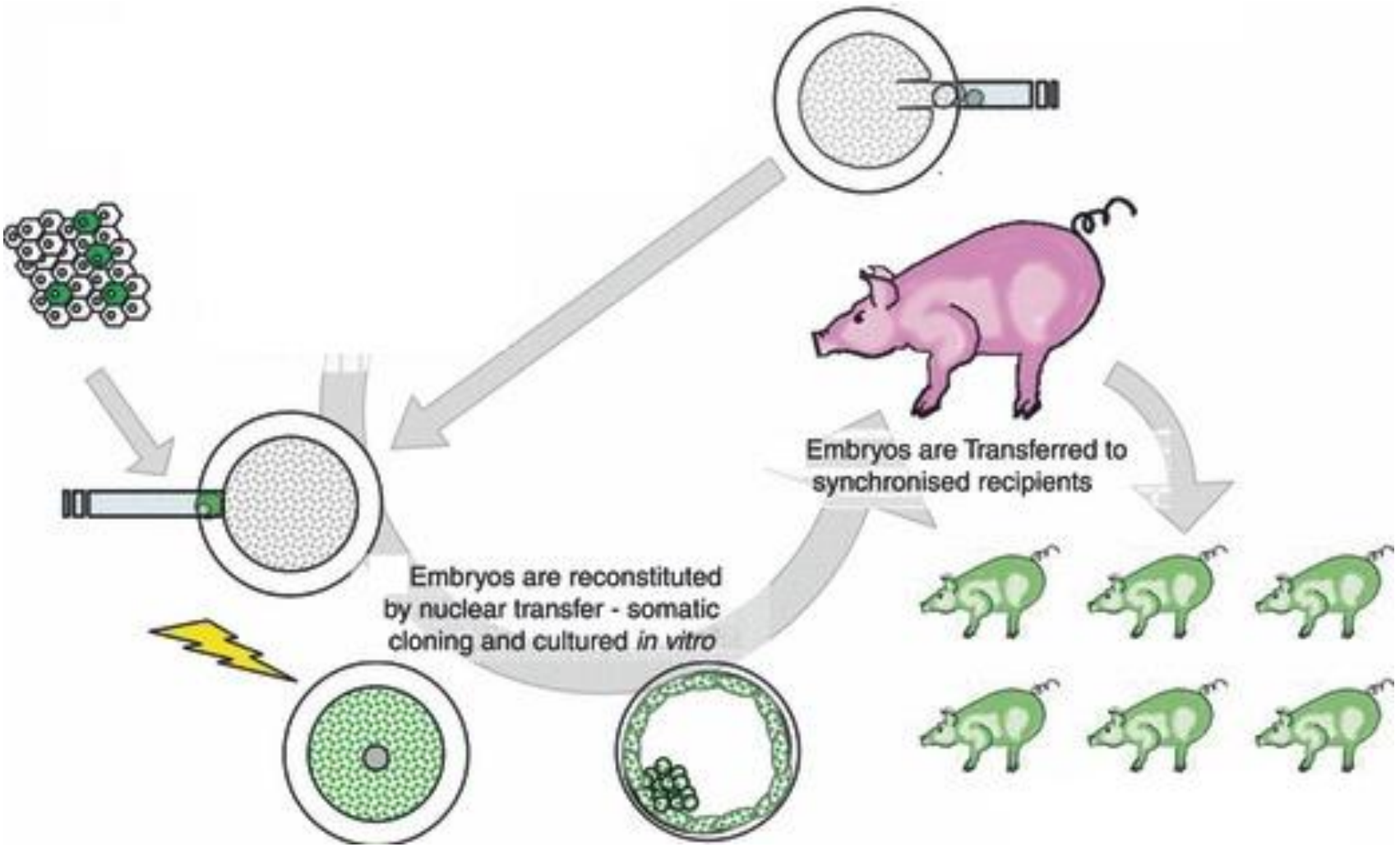
+

Targeting vector



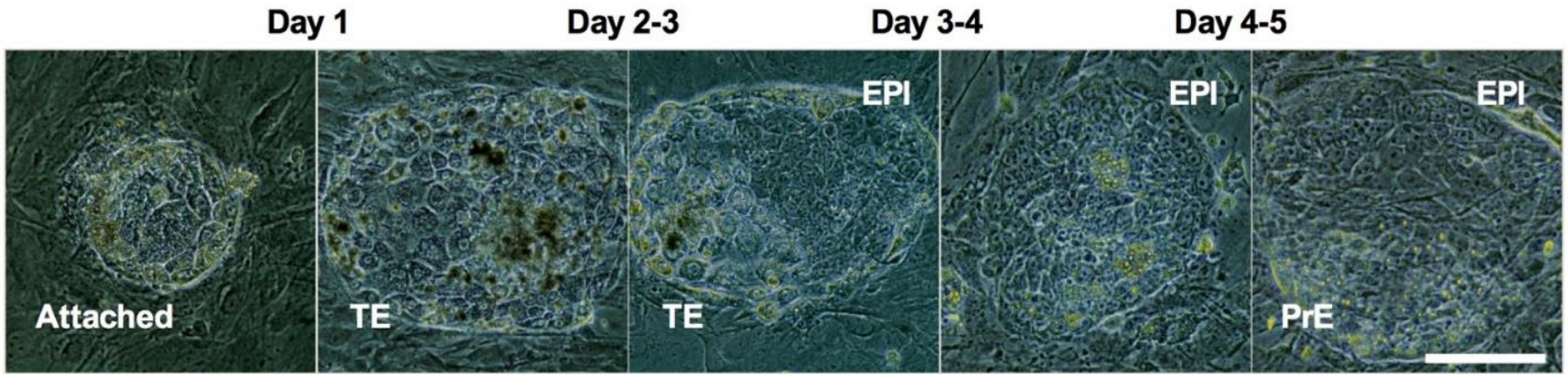


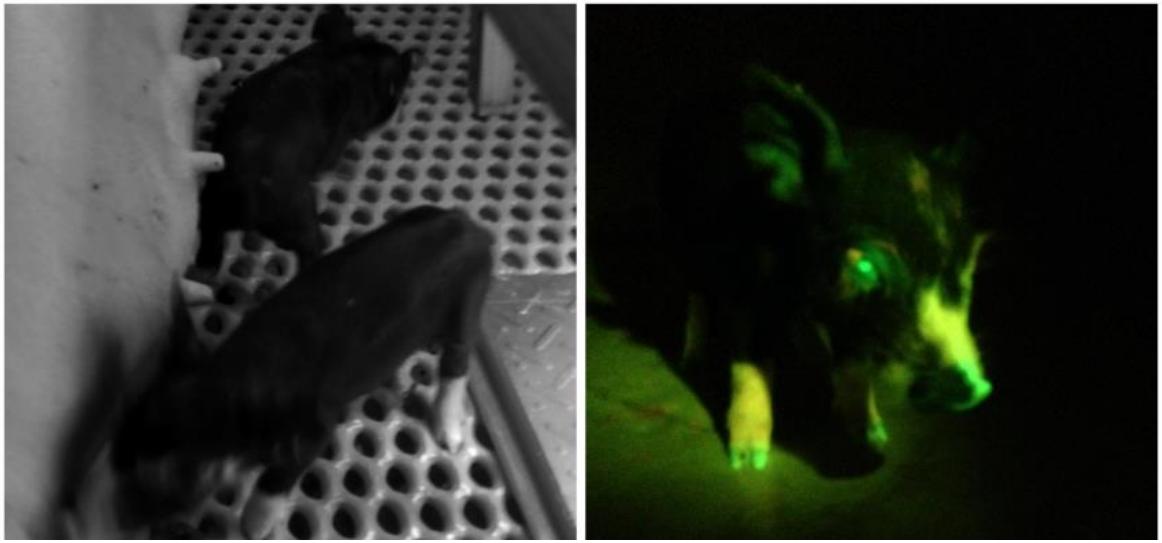
Generation of live pigs by embryonic fibroblasts



Modified from: Galli et al., Xenotransplantation 2010

Extraembryonic (XEN) cells established from porcine embryos

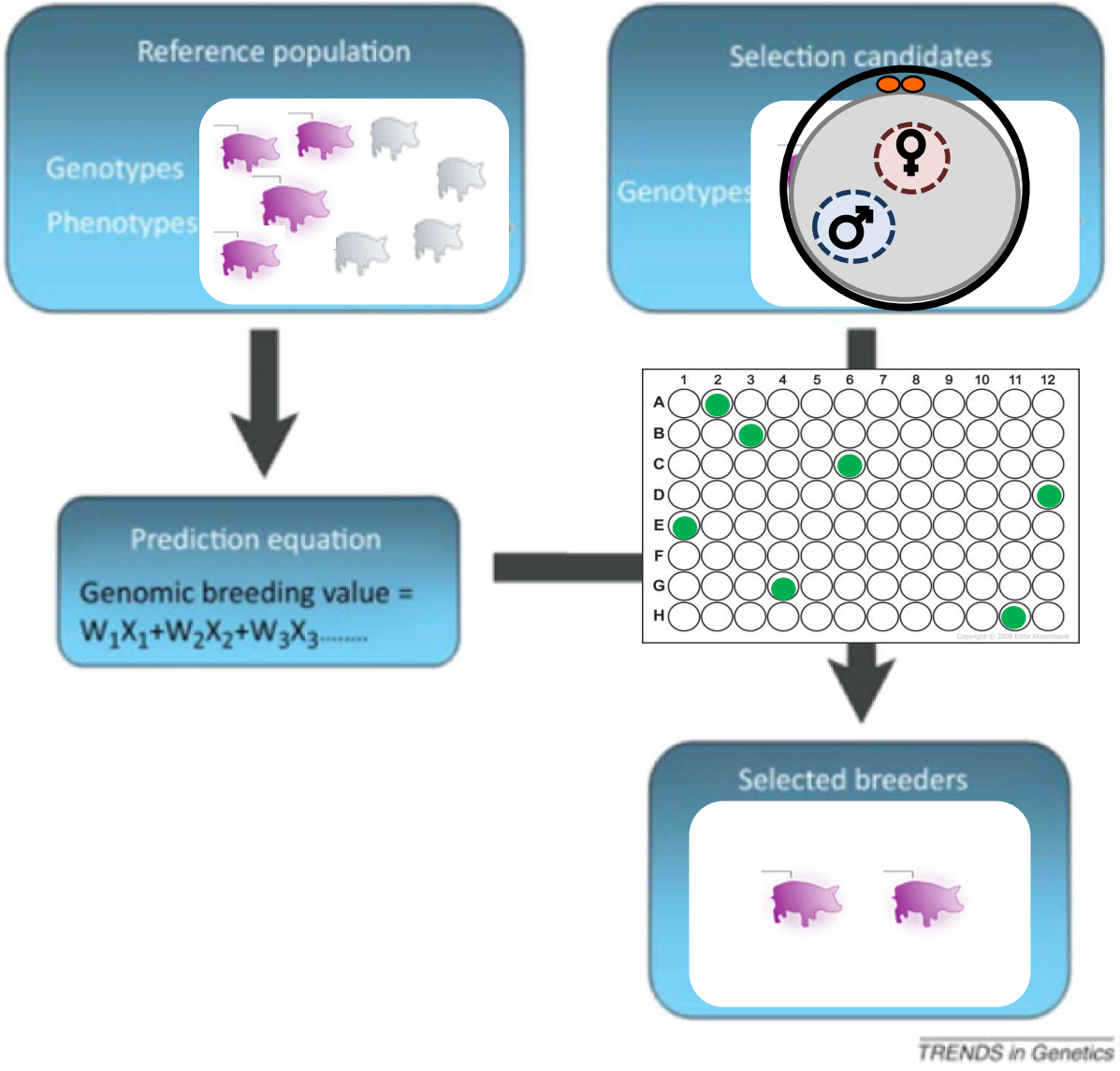




Park et al., unpublished

Where do we go from here ?

Other applications: In vitro breeding (Genetic selection in vitro)



Summary

- Use the methodologies for genomic selection (in vitro breeding)
- The in vitro methodologies will permit for multiplexing edits, before generating live offspring.

Acknowledgements

Postdocs:

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- Jessica Martin
- Jessica Bridge

Graduate students:

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- Laramie Pence
- Timothy Sheets

Announcement

September 23-25, 2017

6th Swine in Biomedical Research Conference

Baltimore, MD September 23-25, 2017

Organizing Chair: Dr. Bhanu Telugu